

## ONLINE SUPPLEMENTAL MATERIAL

### **Treatment of Primary Aldosteronism Increases Plasma Epoxyeicosatrienoic Acids**

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**Short Title: Aldosterone decreases epoxyeicosatrienoic acids**

**Table S1. qPCR Primer Sequences**

<b>Gene</b>	<b>Primers</b>	<b>PCR conditions</b>
<b><i>Ephx2</i></b>	Forward 5' CTGGCCCTCCCCTCTATCG 3' Reverse 5' GCACCAAGCAGGAAGTCTCT 3'	95°C for 3 min, 1 cycle 95°C for 30s } 55°C for 30s } 40 cycles 72°C for 30s }
<b><i>GAPDH</i></b>	Forward: 5' GTGGTGAAGCAGGCATCTGA 3' Reverse: 5' AGGAGACAACCTGGTCCTCA 3'	According to the PCR conditions of respective gene.

**Table S2: Baseline characteristics of patients with primary aldosteronism grouped by subsequent treatment.**

<b>Measure</b>	<b>Adrenalectomy</b> (n=6)	<b>MRA</b> (n=3)	<b>All</b> (n=9)
Age (years)	52.4 ± 7.8	44.0 ± 12.2	49.6 ± 9.6
Gender, female (%)	3 (50%)	1 (33%)	4/9 (44%)
Race, n (%)			
White	5/6 (83%)	1/3 (33%)	6/9 (67%)
African American	1/6 (17%)	2/3 (67%)	3/9 (33%)
Body mass index (kg/m <sup>2</sup> )	26.5 ± 2.9	33.9 ± 6.3	29.0 ± 5.3
Systolic blood pressure (mmHg)	139.0 ± 16.4	144.3 ± 5.5	140.8 ± 13.5
Diastolic blood pressure (mmHg)	86.0 ± 7.2	82.3 ± 10.3	84.8 ± 7.9
Heart rate (bpm)	66.0 ± 12.0	73.0 ± 7.2	68.3 ± 10.8
PAC (ng/dL)	39.3±40.7	21.0±3.8	33.2±33.5
PRA (ng/mL/hr)	0.23 ± 0.18	0.40 ± 0.14	0.28 ± 0.18
PAC:PRA ratio (ng/dL per ng/ml/hr)	197.9±81.8	41.2±13.5	153.2±101.7

Continuous variables are reported as mean ± SD

MRA, mineralocorticoid receptor antagonist; PAC, plasma aldosterone concentration; PRA, plasma renin activity

**Table S3: Characteristics of patients with primary aldosteronism prior to and three to twelve months following treatment.**

<b>Measure</b>	<b>Pre-treatment</b>	<b>Treated</b>	<b>P-value</b>
Weight (kg)	87.7 ± 23.0	89.3 ± 22.1	0.25
SBP (mmHg)	131.1 ± 10.8	124.3 ± 23.1	0.43
DBP (mmHg)	81.5 ± 8.2	77.1 ± 15.0	0.36
HR (bpm)	61.3 ± 7.2	59.6 ± 6.2	0.30
Serum Potassium (mmol/L)	3.7 ± 0.2	4.1 ± 0.4	0.06
Creatinine (mg/dL)	1.09 ± 0.05	1.21 ± 0.13	0.35
Aldosterone (ng/dL)	20.41 ± 15.20	6.16 ± 3.68	0.02
11-deoxycorticosterone (pg/mL)	47.4±34.1	16.6±19.3	0.02
Cortisol (µg/dL)	9.7±2.7	9.1±2.6	0.57
ACTH (pg/mL)	40.4 ± 7.5	46.6 ± 16.0	0.25
Glucose (mg/dL)	93.2 ± 10.8	94.0 ± 10.5	0.82
Insulin (µU/mL)	10.2 ± 2.7	13.9 ± 6.2	0.13
C-peptide (ng/mL)	1.6 ± 0.5	2.1 ± 0.7	0.13
Body fat (%)	35.3 ± 6.5	36.7 ± 6.5	0.06
Body fat, Gynoid (%)	39.0 ± 9.0	39.2 ± 9.9	1.0
Body fat, Android (%)	40.4 ± 9.2	43.2 ± 9.7	0.02

Continuous variables are reported as mean ± SD.

SBP indicate systolic blood pressure, DBP diastolic blood pressure, HR heart rate

**Table S4. Plasma sEH activity in patients with primary aldosteronism, determined by substrate incubation**

Measure	Pre-Treatment	Post-Treatment	Within-Subject $\Delta$	P-value
sEH Activity (11,12-DHET)	1.86 $\pm$ 0.63	1.80 $\pm$ 0.67	-0.06 $\pm$ 0.38	0.91
sEH Activity (12,13-DiHOME)	2.15 $\pm$ 0.97	2.51 $\pm$ 1.42	0.35 $\pm$ 1.11	0.36
sEH Activity (14,15-DHET)	3.21 $\pm$ 1.18	2.88 $\pm$ 1.12	-0.33 $\pm$ 1.12	0.82
sEH Activity (14,15-DiHOME)	2.69 $\pm$ 1.16	2.60 $\pm$ 1.13	-0.09 $\pm$ 0.72	0.82
sEH Activity (8,9-DHET)	0.66 $\pm$ 0.09	0.80 $\pm$ 0.09	0.14 $\pm$ 0.12	0.027

Wilcoxon signed-rank test. Results are mean $\pm$ SD

**Table S5. Plasma sEH activity in patients with primary aldosteronism, estimated by endogenous plasma eicosanoid ratios**

Measure	Pre-Treatment	Post-Treatment	Within-Subject $\Delta$	P-value
Total DHET:(DHET+EET)	0.37 $\pm$ 0.08	0.33 $\pm$ 0.07	-0.04 $\pm$ 0.06	0.074
8,9-DHET:(DHET+EET)	0.40 $\pm$ 0.05	0.41 $\pm$ 0.04	0.01 $\pm$ 0.07	0.82
11,12-DHET:(DHET+EET)	0.43 $\pm$ 0.06	0.41 $\pm$ 0.04	-0.03 $\pm$ 0.06	0.20
14,15-DHET:(DHET+EET)	0.27 $\pm$ 0.14	0.23 $\pm$ 0.13	-0.04 $\pm$ 0.09	0.16
Total DiHOME:(DiHOME+EpOME)	0.39 $\pm$ 0.08	0.38 $\pm$ 0.10	-0.01 $\pm$ 0.06	0.57
9,10-DiHOME:(DiHOME+EpOME)	0.39 $\pm$ 0.07	0.38 $\pm$ 0.09	-0.02 $\pm$ 0.06	0.43
12,13-DiHOME:(DiHOME+EpOME)	0.38 $\pm$ 0.10	0.37 $\pm$ 0.11	-0.01 $\pm$ 0.06	0.91

Wilcoxon signed-rank test. Results are mean $\pm$ SD

**Table S6: Correlation between epoxyeicosatrienoic acid (EET) concentrations and measures and adrenal steroids.**

Measure	14,15-EET	11,12-EET	8,9-EET	Total EETs	14,15-DHET:(DHET+EET)
ACTH	$\rho=-0.56$ $P=0.11$	$\rho=-0.5$ $P=0.17$	$\rho=-0.51$ $P=0.16$	$\rho=-0.56$ $P=0.11$	$\rho=-0.5$ $P=0.17$
11-deoxycortisol	$\rho=-0.56$ $P=0.11$	$\rho=-0.37$ $P=0.34$	$\rho=-0.24$ $P=0.67$	$\rho=-0.49$ $P=0.17$	$\rho=-0.07$ $P=0.92$
DOC	$\rho=-0.21$ $P=0.71$	$\rho=0.01$ $P=0.99$	$\rho=0.01$ $P=0.99$	$\rho=-0.14$ $P=0.83$	$\rho=-0.13$ $P=0.85$
Cortisone	$\rho=-0.18$ $P=0.77$	$\rho=-0.01$ $P=0.99$	$\rho=0.01$ $P=0.99$	$\rho=-0.09$ $P=0.92$	$\rho=-0.07$ $P=0.92$
18OH-Cortisol	$\rho=-0.1$ $P=0.89$	$\rho=-0.39$ $P=0.32$	$\rho=-0.45$ $P=0.22$	$\rho=-0.18$ $P=0.77$	$\rho=-0.05$ $P=0.93$
Testosterone	$\rho=-0.44$ $P=0.25$	$\rho=-0.3$ $P=0.51$	$\rho=-0.12$ $P=0.86$	$\rho=-0.39$ $P=0.32$	$\rho=0$ $P=1.00$
Progesterone	$\rho=-0.04$ $P=0.95$	$\rho=0.17$ $P=0.78$	$\rho=0.14$ $P=0.83$	$\rho=0.05$ $P=0.93$	$\rho=0.07$ $P=0.92$
17OH-Progesterone	$\rho=-0.11$ $P=0.87$	$\rho=0.24$ $P=0.65$	$\rho=0.21$ $P=0.71$	$\rho=0$ $P=1.00$	$\rho=-0.17$ $P=0.79$
Androstenedione	$\rho=-0.18$ $P=0.77$	$\rho=0.16$ $P=0.80$	$\rho=0.25$ $P=0.65$	$\rho=-0.07$ $P=0.92$	$\rho=-0.14$ $P=0.83$

*P*-values are adjusted for multiple testing according to Benjamini and Hochberg.  $\rho$  indicates Spearman's rank correlation  $\rho$ . Results for the adrenal steroids Aldosterone, Corticosterone, and Cortisol are presented in the main text.

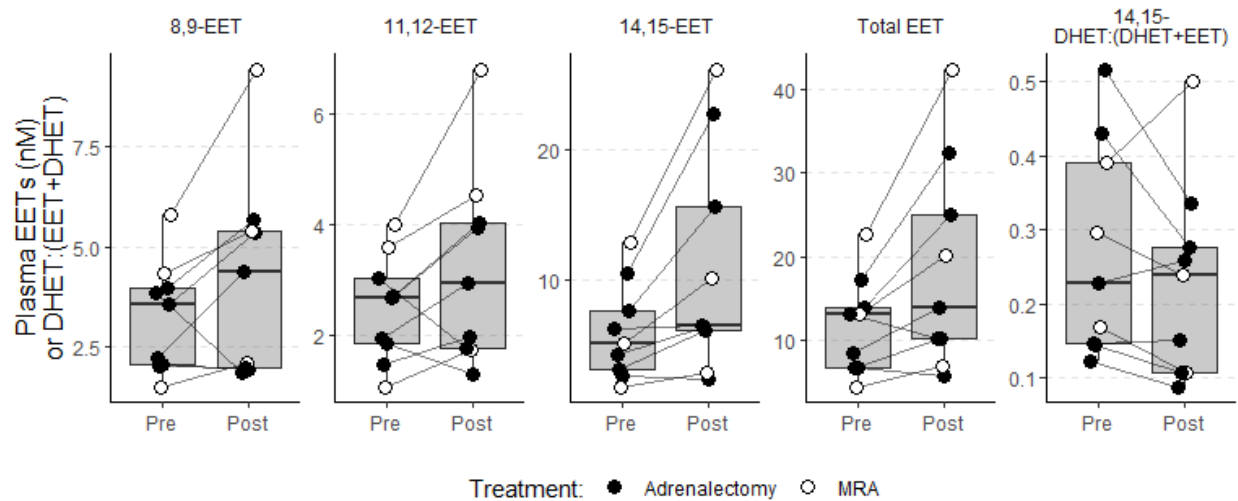
ACTH, adrenocorticotrophic hormone; DOC, 11-deoxycorticosterone; 18OH-Cortisol, 18-hydroxycortisol; 17OH-Progesterone; 17 $\alpha$ -hydroxyprogesterone

**Table S7. Correlations between ex vivo sEH activity and DHET:(EET+DHET) molar ratios.**

Measure	sEH Activity (14,15-DHET)	sEH Activity (11,12-DHET)	sEH Activity (8,9-DHET)	sEH Activity (12,13-DiHOME)	sEH Activity (14,15-DiHOME)
Total DHET:(DHET+EET)	$\rho=-0.71$ <b><math>P=0.008</math></b>	$\rho=-0.54$ $P=0.076$	$\rho=-0.2$ $P=0.57$	$\rho=-0.51$ $P=0.10$	$\rho=-0.42$ $P=0.16$
Total DiHOME:(DiHOME+EpOME)	$\rho=0.46$ $P=0.13$	$\rho=0.56$ $P=0.066$	$\rho=0.22$ $P=0.54$	$\rho=0.47$ $P=0.13$	$\rho=0.48$ $P=0.12$
8,9-DHET:(DHET+EET)	$\rho=0.06$ $P=0.86$	$\rho=0.12$ $P=0.74$	$\rho=-0.01$ $P=0.96$	$\rho=0.18$ $P=0.60$	$\rho=0.06$ $P=0.86$
11,12-DHET:(DHET+EET)	$\rho=-0.22$ $P=0.55$	$\rho=0.18$ $P=0.60$	$\rho=0.1$ $P=0.79$	$\rho=0.2$ $P=0.57$	$\rho=0.22$ $P=0.54$
14,15-DHET:(DHET+EET)	$\rho=-0.7$ <b><math>P=0.008</math></b>	$\rho=-0.68$ <b><math>P=0.012</math></b>	$\rho=-0.19$ $P=0.60$	$\rho=-0.62$ <b><math>P=0.031</math></b>	$\rho=-0.56$ $P=0.065$
9,10-DiHOME:(DiHOME+EpOME)	$\rho=0.45$ $P=0.13$	$\rho=0.52$ $P=0.090$	$\rho=0.23$ $P=0.54$	$\rho=0.46$ $P=0.13$	$\rho=0.48$ $P=0.12$
12,13-DiHOME:(DiHOME+EpOME)	$\rho=0.47$ $P=0.13$	$\rho=0.6$ $P=0.042$	$\rho=0.3$ $P=0.37$	$\rho=0.48$ $P=0.12$	$\rho=0.4$ $P=0.19$
sEH Activity (14,15-DHET)	-	$\rho=0.55$ $P=0.070$	$\rho=0.07$ $P=0.86$	$\rho=0.61$ <b><math>P=0.037</math></b>	$\rho=0.65$ <b><math>P=0.021</math></b>
sEH Activity (11,12-DHET)	-	-	$\rho=-0.03$ $P=0.93$	$\rho=0.82$ <b><math>P=0.0004</math></b>	$\rho=0.77$ <b><math>P=0.002</math></b>
sEH Activity (8,9-DHET)	-	-	-	$\rho=0.08$ $P=0.84$	$\rho=-0.12$ $P=0.74$
sEH Activity (12,13-DiHOME)	-	-	-	-	$\rho=0.89$ <b><math>P\leq 0.0001</math></b>
sEH Activity (14,15-DiHOME)	-	-	-	-	-

*P*-values are adjusted according to Benjamini and Hochberg.  $\rho$  indicates Spearman's rank correlation  $\rho$ .

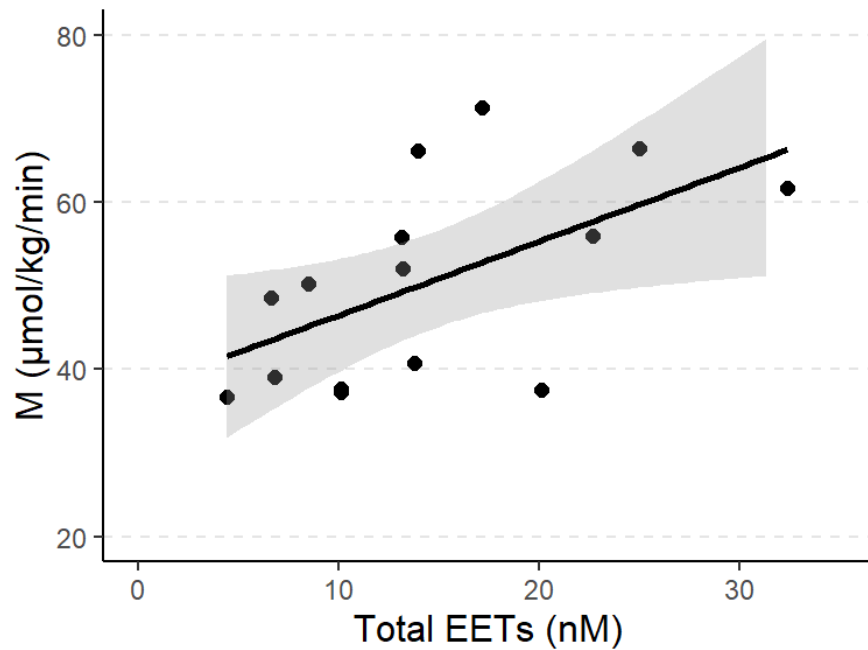
**Figure S1.**



**Figure S1. Plasma total and 14,15-EET regioisomer concentrations increase after treatment of primary aldosteronism.** Soluble epoxide hydrolase activity, estimated as 14,15-EET:(14,15-DHET+14,15-EET) concentration, did not change significantly. Black circles indicate patients who underwent adrenalectomy and open circles indicate those who were treated with a mineralocorticoid receptor antagonist (MRA). 14,15-EET and total EET concentrations increased significantly after treatment.

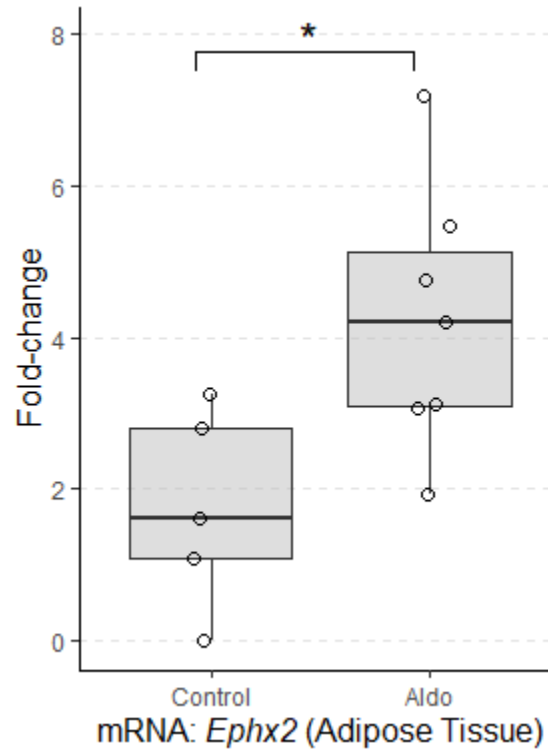


**Figure S2**



**Figure S2. Total EETs directly correlate with insulin sensitivity.** Relationship between total epoxyeicosatrienoic acid (EET) concentrations and insulin sensitivity, as measured by the glucose infusion rate (M) during hyperinsulinemic clamp steady state insulin infusion at a rate of 120 mU/m<sup>2</sup>/min (average achieved insulin concentration of 2,089±466 pmol/L). Data includes results obtained during hyperinsulinemic clamps were before and after treatment of primary aldosteronism. Spearman's rank correlation  $\rho$ : 0.64,  $P=0.027$ .

**Figure S3**



**Figure S3: Aldosterone increases sEH mRNA expression in adipose tissue.**

mRNA expression of *Ephx2* was estimated in adipose tissues collected from saline control (n = 5) and aldosterone treated (n=7) mice via osmotic minipump for 3 days. The traditional  $2^{-\Delta\Delta C_t}$  method was employed to evaluate fold-change *Ephx2* mRNA expression, with GAPDH as the housekeeping gene. \* $P=0.03$  by Wilcoxon rank sum test for difference between treatments, indicating a significant increase in *Ephx2* mRNA expression.